Genetic Equivalence of Putative Duplicate Germplasm Collections Held at CIP and US Potato Genebanks

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ABSTRACT

A common effort among members of the Association of Potato Inter-Genebank Collaborators (APIC) has yielded a global inventory of wild potato genetic resources that is freely accessible to researchers and breeders. In that database there are a number of accessions that originated from distributed progeny of a single original germplasm collection. The logical assumption has been that although these samples are in different locations, they should be genetically equivalent. This study tests this hypothesis by comparing 17 pairs of accessions of 16 different potato species, which are reputed duplicates preserved in the potato genebanks of The International Potato Center (CIP) in Peru and of the U.S.A. (USPG). The RAPD marker analysis revealed that even though the average genetic similarity of reputed duplicates was quite high, there were a few with significant differences. Similarly, SSR markers identified three reputed duplicates that were genetically different. SSRs revealed a loss of markers for some inter-genebank comparisons, a probable indication of genetic drift. Duplicate potato collections between CIP and USPG are in most cases genetically identical. The few exceptions merit further investigation regarding causes and the impact on useful traits.

RESUMEN

Un esfuerzo común entre los miembros de la Asociación de Colaboradores de Bancos de Germoplasma de Papa (APIC) ha dado como resultado un inventario global de recursos genéticos de papa silvestre, de libre acceso a investigadores y mejoradores. En la base de datos existe un número de accesiones que se han generado a partir de la progenie distribuida de la colección original. La suposición lógica ha sido que aunque estas muestras están en diferentes lugares, deberían ser genéticamente equivalentes. Este estudio prueba esta hipótesis al comparar 17 pares de accesiones de 16 especies diferentes de papa, las cuales son consideradas como duplicados mantenidos en el banco de germoplasma del Centro Internacional de la Papa (CIP) en Perú y en los Estados Unidos de América (USPG). El análisis con el marcador RAPD reveló que a pesar de que la similaridad genética de duplicados putativos fue bastante alta, sin embargo, hubo algunos con diferencias significativas. Igualmente los marcadores SSR identificaron tres duplicados putativos genéticamente diferentes. Los SSR revelaron una pérdida de marcadores para algunas comparaciones entre bancos de genes, probable indicación de deriva genética. Los duplicados

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ADDITIONAL KEY WORDS: duplicate collections, genetic drift, potato genebanks, potato germplasm, RAPD markers, SSRs

ABBREVIATIONS: APIC, Association of Potato Intergenebank Collaborators; CIP, The International Potato Center; GS, genetic similarity; GA_3, giberellic acid; RAPD, random amplified polymorphic DNA; SSR, simple sequence repeats; USPG, US Potato Genebank; VIR, Vavilov Institute Potato Genebank

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de las colecciones entre CIP y USPG son, en la mayoría de los casos, genéticamente idénticos. Las pocas excepciones que existen ameritan investigación futura para determinar las causas y su efecto en caracteres de interés.

INTRODUCTION

In recent decades, a number of reports have indicated a progressive reduction and/or deterioration of natural environments that native plant species inhabit. Several factors contributing to this habitat attrition have been documented, ranging from urban expansion to global warming (Rosenzweig 2001; Tilman and Lehman 2001). Reduction of habitats has obviously jeopardized the continued existence of many plant species, and in fact, many of them already face extinction. Conservation of plant species, therefore, has become a priority task. One option to safely conserve and protect plant diversity is to maintain samples of populations collected *in situ* outside of their natural habitats (*ex situ*). For instance, for several decades, potato genebanks worldwide have undertaken the task of collecting and preserving potato populations from different geographical origins (Hanneman 1989). There are nearly 200 different species spread along the American continent (Hijmans and Spooner 2001), which include a wide range of ecological niches and geographical settings; many of these habitats are certainly vulnerable.

Potato genebanks initiated a formal network to exchange information and methods as well as to coordinate research on problems of common interest (Bamberg et al. 1995). One accomplishment has been the creation of a comprehensive database of passport and evaluation records to consolidate information of wild potato species maintained in different potato genebanks. It shows that there was a single, original germplasm collection that was shared and separately multiplied at two or more genebanks (Huaman et al. 2000). The assumption has been that any assessment or characterization gathered at one genebank can be extrapolated to the presumed duplicate accession preserved at another genebank. However, all genebanks do not use the same procedures for seed increase, which might have an influence in modifying the genetic structure of germplasm populations preserved at genebanks (Schoen et al. 1998; Widrlechner et al. 1989). Genetic drift can potentially be generated by deficient sampling, seed contamination, environmental selection, and/or imperfect seed multiplication. For instance, Borner et al. (2000) indicated that genetic drift was observed for one of the wheat accessions maintained and regenerated at the genebank for very long time.

This study represents the second focus of a research project aimed at assessing the true amount of genetic similarity between presumed duplicate accessions held at different potato genebanks. Recently, we reported the findings between the Vavilov Institute potato collection (VIR), St. Petersburg, Russia, and the US Potato Genebank (USPG), Sturgeon Bay, WI, USA (Bamberg et al. 2001). Here we are reporting the results between collections preserved at the International Potato Center (CIP), Lima, Peru, and the USPG.

MATERIALS AND METHODS

Plant Material

Seventeen accessions corresponding to 16 different potato species were identified as duplicated collections maintained at CIP and USPG (Table 1). The selection criteria used to choose these accessions were the availability of seeds and that at least one seed increase occurred at the secondary receiving station. These samples were progeny generated from original populations collected at CIP and then donated to USPG. Therefore, the samples evaluated were derived from seed increase progeny of different samples from the same original population. Table 1 provides identities of these materials. More complete information about the populations used in this study can be accessed through online databases linked to the USPG homepage, http://www.ars-grin.gov/nr6. Sets of 100 botanical seeds were sent from CIP to the USPG near Sturgeon Bay, WI. Each accession was propagated in two replicates of 50 seeds each. The treatment of the materials was performed as identically as possible and at the same time. Seeds were immersed in 2000 ppm of GA_3 for 24 h, dispersed over potting medium in 10-cm clay pots and, then covered with a thin layer of Vermiculite. Leaf tissue was sampled from each plant and bulked for DNA extraction.

DNA Isolation

Samples of DNA were isolated from replicate sets of 27 plants from each accession (Table 1). The samples were fresh young leaf tissue that was bulked from each population to proceed with the DNA extraction. The method used to isolate DNA was a procedure modified from Williams et al. (1994).

The extracted DNA was stored in TE 1X buffer (Promega, Madison, WI) and at -20 C. Samples were quantified using a TKO 100 Mini-Fluorometer (Hoefer Scientific Supplies, San Francisco, CA).

RAPD and SSR Marker Assay

Primers that represent random 10 nucleotide sequences were obtained from Operon Technologies (Alameda, CA) and used in the RAPD assay. PCR amplifications were performed in a 15-µL reaction as described in del Rio et al. (1997). All the DNA fragments were separated by electrophoresis in 1.6% agarose gels and visualized by ethidium bromide staining.

Ten primers corresponding to five simple sequence repeat (SSR) markers previously characterized in potato by Milbourne et al. (1998) (Table 2) were synthesized on a Perkin Elmer Applied Biosystems Synthesizer (Model 3948, Norwalk, CT) at the DNA Synthesis and Sequencing Facility of the University of Wisconsin Biotechnology Center (Madison, WI) and used for PCR amplification. PCR was performed in 15-µL reaction volumes containing the same reagents as specified for RAPD assays, but using 15 ng of genomic DNA and 75 pmoles of each primer of the pair. All PCR amplifications were performed in a Perkin Elmer Applied Biosystems Cetus DNA Thermal Cycler 9700 (Norwalk, CT) programmed for 95 C for 1 min, 65 C 1 min, 72 C 1 min 30 sec for one cycle, then 95 C 1 min, 55 C 1 min, 72 C 1 min 30 sec, repeated for a total of 30 cycles and then maintained at 4 C. Table 2 shows the primer sequences and target repeats. All SSR products were fractionated through electrophoresis in 5.0% super fine resolution (SFR) agarose gels (Amresco, Solon, OH) and visualized by ethidium bromide.

TABLE 1—*Reputed duplicates in CIP and USPG used in this study.*

Collector's number	CIP code (CIP)	USPG Code (PI)	Species name (*S.*=*Solanum*)	Species abbreviation
OCH 12092	761057	498200	*S. acaule* ssp. *acaule*	acl pne[1]
OCH 12090	761453	498199	*S. albicans*	alb
OCH 14142	761888	498223	*S. bulbocastanum*	blb
OCH 13009	761466	498214	*S. blancogaldosii*	blg
OCH 11954	761364	568921	*S. boliviense*	blv
OCH 13637	761690	568922	*S. buesii*	bue
OCH 11915	761030	473458	*S. circaeifolium*	crc cap[2]
OCH 11814	761282	568969	*S. candolleanum*	cnd
OCH 13401	761609	498228	*S. colombianum*	col
OCH 11619	761238	498243	*S. huancabambense*	hcb
OCH 14208	761928	604098	*S. iopetalum*	iop
OCH 11617	761236	498253	*S. lignicaule*	lgl
OCH 12096	761018	498267	*S. multiinterruptum*	mtp
OCH 7613	761113	473466	*S. raphanifolium*	rap1[3]
OCH 13645	761694	498278	*S. raphanifolium*	rap2[3]
OCH 14135	761884	498287	*S. stoloniferum*	sto
OCH 12001	761007	498290	*S. tarijense*	tar

[1]Previously known as *Solanum acaule* subsp. *punae*. Currently accepted nomenclature is *Solanum acaule* subsp. *acaule* (12-Feb-2002).
[2]Currently accepted nomenclature *Solanum circaeifolium* var. *capsicibaccatum*.
[3]Numbers added to the abbreviation to differentiate accessions from the same species.

TABLE 2—*List of microsatellites (SSR markers) used to assess the potato populations used in this study.*

SCRI code	Repeat Motif	Orientation	Sequence	Chromosome
STM0001	(TG)4 (TC)2 (TG)5	F	AGTATTCAACCCATTGACTTGGA	VI
		R	TAGACAAGCCAAGCTGGAGAA	
STM0003	(AC)9 (AT)9	F	GGAGAATCATAACAACCAG	XII
		R	AATTGTAACTCTGTGTGTGTG	
STM0004	(AC)9 (AT)7 (AC)5	F	CGAGGGCGTAAACTCATGATA	VII
		R	AGGTTATTGTGGACACAGTCTTCA	
STM0006	(AC)14 (AT)5	F	GAAGTTGACATTGAGCCC	N/A
		R	GGATATCCATTCTTAGATGCA	
STM0007	(AC)9	F	GGACAAGCTGTGAAGTTTAT	XII
		R	AATTGAGAAAGAGTGTGTGTG	

N/A Microsatellite has not been assigned to any chromosome.

Statistical Analysis

Samples from different genebanks would be declared significantly different when their observed genetic similarity (GS) had less than a 5% probability of occurring by chance in samples from a binomial distribution defined by the observed replicate GS (duplicate samples from the same seedlot).

RESULTS

The plant materials exhibited some differences in their size and in the rate of germination in the greenhouse. The visual examination of the plants indicated that 10 accessions had seedlings that looked similar for both genebanks. In four cases USPG plants looked smaller than CIP plants and in three cases CIP plants were smaller.

TABLE 3—*RAPD marker comparison between presumed duplicates at CIP and USPG.*

	Within CIP populations		Within USPG populations		Between Genebanks		Genetic Similarity		
Species	Loci Shared	Loci Different	Loci Shared	Loci Different	Loci Shared	Loci Different	Within CIP	Within USPG	Between Genebanks
acl pne	102	0	102	0	102	0	1.000	1.000	1.000
alb	104	0	104	0	104	0	1.000	1.000	1.000
blb	99	0	99	0	99	0	1.000	1.000	1.000
blg	97	0	102	0	97	5	1.000	1.000	0.951*
blv	101	0	102	0	101	2	1.000	1.000	0.985
bue	90	0	90	0	90	0	1.000	1.000	1.000
crc cap	108	0	108	0	108	0	1.000	1.000	1.000
cnd	91	0	86	0	85	5	1.000	1.000	0.944*
col	82	0	83	0	82	2	1.000	1.000	0.981
hcb	83	0	83	0	83	0	1.000	1.000	1.000
iop	90	0	90	0	90	0	1.000	1.000	1.000
lgl	83	0	83	0	83	0	1.000	1.000	1.000
mtp	97	0	99	0	97	2	1.000	1.000	0.984
rap1	93	0	94	0	93	2	1.000	1.000	0.983
rap2	99	0	99	0	99	0	1.000	1.000	1.000
sto	90	0	90	0	90	0	1.000	1.000	1.000
tar	91	0	91	0	91	0	1.000	1.000	1.000
Average	94.12	0	94.41	0	93.77	1.06	1.000	1.000	0.996

*Significantly different at $P \leq 0.05$.

The genetic assessment of genebank pairs was based on RAPD and SSR markers. For RAPD markers, an average of 94 markers was used for each inter-genebank comparison (Table 3). The total number of bands evaluated for all the inter-genebank comparisons were 1,612 from which 1,594 (98.9%) were matches and 18 (1.1%) mismatches. The marker presence or absence for each DNA sample was considered equivalent to the presence or absence of a dominant allele at a random locus. The measure of relatedness was GS calculated as the percentage loci with matching RAPD markers. The average GS between inter-genebank populations was 99.6%; the lowest GS detected was 94.4% between populations of *Solanum candolleanum* (PI 568969) and the highest GS was 100.0% found 11 times. A total of two inter-genebank comparisons (blg, cnd) were found to be significantly different (Table 3).

The replicate GS (of duplicate samples from the same seedlot) was 100% in all 34 observations (17 populations x 2 genebanks). Thus, since observed experimental error was zero, all of the six inter-genebank GS listed in Table 2 that are less than 1.000 are statistically significant. However, results of past experiments done with similar materials and methods (Bamberg et al. 2001; del Rio and Bamberg 2000, 2004) suggest that the typical observed average GS of replicates is about 99.7%. If 17 random samples of size = 93 bands are taken from a binomial distribution where $P = 0.997$, there is about a 5% chance of any sample being as low as 90/93 = GS of 0.968. Thus, a more conservative analysis might declare only inter-genebank GS lower than 96.8% to represent significant divergence of the samples from the two genebanks, and this is true of only two of the populations (Table 3).

For SSRs, 27 markers were detected for the 16 potato species evaluated. In some species, certain markers were not detected (Table 4), indicating that they had been lost. The species *Solanum blancogaldosii*, *Solanum buesii* and *S. candolleanum* showed such differences.

DISCUSSION

Van Hintum (2000) pointed out that estimating levels of duplicate germplasm within and between genebanks is of high importance for judging the efficiency of *ex situ* conservation efforts. The present study complements that interest and provides new insights on the integrity of potato inter-genebank duplicates. Our results revealed that although a few pairs exhibited lower GS values than those anticipated from duplicate sampling, most CIP and USPG duplicate holdings are very similar. These findings differ with the previous study between the VIR (Russia) and USPG collections where most reputed duplicates showed small but significant differences (Bamberg et al. 2001).

TABLE 4—*Number of SSR markers amplified in CIP-USPG comparisons of reputed duplicate populations.*

	SSR PRIMER									
	STM0001		STM0003		STM0004		STM0006		STM0007	
Species	CIP	USPG	CIP	USPG	CIP	USPG	CIP	USPG	CIP	USPG
acl pne	1	1	1	1	1	1	1	1	2	2
alb	1	1	1	1	1	1	1	1	1	1
blb	1	1	1	1	0	0	0	0	2	2
blg	2	2	1	1	1	1	1	1	1	2*
blv	2	2	1	1	1	1	0	0	2	2
bue	1	1	0	0	1	1	1	2*	1	1
crc cap	1	1	0	0	0	0	0	0	1	1
cnd	2	2	1	1	1	1	1	1	2	1*
col	2	2	1	1	0	0	1	1	1	1
hcb	2	2	0	0	1	1	1	1	1	1
iop	2	2	0	0	1	1	1	1	2	2
lgl	2	2	0	0	1	1	1	1	1	1
mtp	2	2	0	0	0	0	1	1	1	1
rap1	0	0	0	0	1	1	1	1	2	2
rap2	0	0	0	0	1	1	1	1	2	2
sto	2	2	0	0	0	0	2	2	2	2
tar	1	1	0	0	1	1	1	1	1	1

*SSR marker variation occurred.

Interpretation of CIP : USPG reputed duplicate population comparisons
0:0 = no marker in either population
1:1 = the same single allele in both populations
2:2 = the same two alleles in both populations
1:0 or 0:1 = one allele present only in one population (none such cases)
1:2 or 2:1 = one allele in common in both populations and one more allele found only in one of the populations (three such cases)

An important foundation of this study was to verify that two samples of individuals from the same population will have very little variation (will always appear to be nearly identical). Virk et al. (1995) emphasized that reproducible genetic profiles of samples originated from the same population are critical for making consistent associations. Our GS assessment of replicate samples found that samples taken from the same population within each genebank were categorized as identical by the molecular markers (Table 3). Therefore, the sampling approach used let the markers unequivocally tag the populations and implied that sampling variation was not a factor explaining genetic differentiation. These findings are in agreement with our previous studies using similar sampling methods (Bamberg et al. 2001; del Rio and Bamberg 2000).

Germplasm management is complex and often labor intensive. Each genebank executes its activities based on its own routines, needs, and resources; however, some of these activities include possibilities of genetic attrition. For example, Widrlechner et al. (1989) reported that deficiencies in the management, such as inadequate sampling in selecting parents, contamination of seed or pollen, unintentional selection of individuals, and environmental bottleneck, could risk the genetic integrity of germplasm. Any or combinations of these factors could have been involved in the few inter-genebank genetic differences found in this study. These differences were detected with both molecular markers (Tables 3 and 4). RAPD markers located disparity from a large-scale view, when overall genome assessment was done, while SSRs detected specific variation at different loci. In either case, finding differentiation between duplicate samples invites the question of how it took place. It is difficult to speculate on causes, however, since details of the differences in how accessions had been grown, multiplied, and stored were not systematically controlled or recorded at either genebank (Bamberg et al. 2001).

Differences in germination and seedling selection are one possible cause of genetic drift. Some species received from CIP were not used because they did not germinate at all in the USPG facilities (acg, ber, bcp, dms, mga), while some of the populations used showed inter-genebank differences (alb, blg, bue, cap, col, mtp, tar) in number and vigor of sprouted

seedlings. But these were not the species that exhibited genetic differentiation (blv, cnd, rap). We know that de-selection of smaller seedlings at transplanting does not cause genetic drift in inbreeding species populations at USPG (Bamberg and del Rio 2006), but do not know if that factor is influential in other species germinated at other genebanks without systematic tests.

The assessment of SSR markers revealed 27 markers expressed among the 16 species. In most cases, SSR markers found that duplicate germplasm is equivalent between genebanks. In some cases, these markers were capable of discriminating between reputed duplicates, suggesting that genetic drift (loss of alleles) has taken place. Two of the three species exhibiting SSR genetic variation (blg, cnd) were also the ones with significant differentiation estimated with RAPDs, which confirms that these accessions underwent drift. The third comparison (bue) showed one SSR marker was lost even though all the RAPD markers were present in both duplicate populations (Tables 3 and 4). Although SSRs are co-dominant, the bulking used here makes them and RAPDs similarly informative in this experiment—i.e., we can only determine if SSR alleles are present or absent, not use them to assess more subtle changes in allele frequency or population structure.

The loss of genetic diversity is an important concern in captive populations. The effects of reduction in diversity differ depending on the amount the diversity is reduced and the type of genetic bottleneck (i.e., number of generations, severity of environmental effects). Small population size may lead to loss of variation and subsequent loss of evolutionary potential (Ellstrand and Elam 1993). A reduced parental population size during seed increase (a form of small population size) could alter the genetic structure and resulting loss of alleles. Antonova et al. (2001) reported that SSR markers detected genetic changes in long-term preserved potato cultivars maintained by the German and Russian genebanks. They hypothesized that differentiation could have been caused by different factors such as the use of growth retardants, environmental bottleneck, inclusion of a cultivar with a genetic modification, instability of the cultivar, mixing of cultivars, and/or mistakes in the conservation methods. In del Rio et al. (1997), we found that genetic drift usually does not arise during seed increase, but one occurrence was reported in *Solnaum jamesii* (a diploid oucrosser, PI 458423.1978). Another study found that the first seed increase alters allelic frequencies modifying the original population structure of recently collected plants (del Rio and Bamberg 2003). However, these studies concluded that although seed increase changes population structure, it usually does not increase the risk of vulnerability since all alleles are maintained.

This comparison of CIP and USPG duplicates demonstrates that RAPDs and SSRs are valuable tools to track allelic vulnerability and to identify potential genetic drift. From a practical standpoint, this result cautions potato genebank managers that diversity might be at risk for some germplasm. When drift is detected, it can be used to evaluate the protocols and conditions of seed increase and management that might be the cause.

In summary, the comparison of inter-genebank reputed duplicates of CIP and USPG collections found that although a few accessions experienced some genetic differentiation, the majority kept their genetic similarity over years of conservation and genebank handling. Thus, duplicate germplasm shared by CIP and USPG may usually be considered duplicates for evaluation, research, and breeding purposes.

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LITERATURE CITED

Antonova OY, LI Kostina, T Gavrilenko, K Schüler and R Thieme. 2001. Proof of long-term stored potato germplasm by use of molecular markers *In:* Band 22: Rudolf Mansfeld and Plant Genetic Resources. Proceedings of a symposium dedicated to the 100th Birthday of Rudolf Mansfeld, Gatersleben, Germany. pp 192-197.

Bamberg JB and AH del Rio. 2006. Seedling transplant selection does not cause genetic shifts in potato genebank populations. Crop Sci 46:424-427.

Bamberg JB, Z Huaman and R Hoekstra. 1995. International cooperation in potato germplasm. *In:* International Germplasm Transfer: Past and Present. CSSA Special Publication 23.

Bamberg JB, S Kiru and AH del Rio. 2001. RAPD comparison of reputed duplicate populations in the VIR and US Potato Genebanks. Amer J Potato Res 78:365-369.

Borner A, S Chebotar and V Korzun. 2000. Molecular characterization of the genetic integrity of wheat (*Triticum aestivum* L.) germplasm after long-term maintenance. Theor Appl Genet 100:494-497.

del Rio AH, JB Bamberg and Z Huaman. 1997. Assessing changes in the genetic diversity of potato gene banks. 1. Effects of seed increase. Theor Appl Genet 95:191-198.

del Rio AH and JB Bamberg. 2000. RAPD markers efficiently distinguish heterogeneous populations of wild potato (*Solanum*). Genet Res Crop Evol 47:115-121.

del Rio AH and JB Bamberg. 2003. The effect of genebank seed increase on the genetics of recently collected potato (*Solanum*) germplasm. Amer J Potato Res 80:215-218.

del Rio AH and JB Bamberg. 2004. Geographical parameters and proximity to related species predict genetic variation in the inbred potato species *Solanum verrucosum* Schlechtd. Crop Sci 44:1170-1177.

Ellstrand NC and DR Elam. 1993. Population genetic consequences of small population size: Implications for Plant Conservation. Ann Rev Ecol Syst 24:217-242.

Hanneman RE. 1989. The potato germplasm resource. Am Potato J 66:655-667.

Hijmans RJ and DM Spooner. 2001. Geographic distribution of wild potato species. Am J Bot 88:2101-2112.

Huaman Z, R Hoekstra and JB Bamberg. 2000. The Inter-genebank Potato Database and the dimensions of available wild potato germplasm. Amer J Potato Res 77:353-362.

Milbourne D, RC Meyer, AJ Collins, LD Ramsay, C Gebhardt and R Waugh.1998. Isolation, characterization and mapping of simple sequence repeat loci in potato. Mol Gen Genet 259:233–245.

Rosenzweig ML. 2001. Loss of speciation rate will impoverish future diversity. Proc Natl Acad Sci 98:5404-5410.

Schoen DJ, JL David and TM Bataillon. 1998. Deleterious mutation and the regeneration of plant genetic resources. Proc Natl Acad Sci 95:394-399.

Tilman D and C Lehman. 2001. Human-caused environmental change: Impacts on plant diversity and evolution. Proc Natl Acad Sci 98:5433-5440.

van Hintum TJL. 2000. Duplication within and between germplasm collections. III. A quantitative model. Genet Res Crop Evol 47:507-513.

Virk PS, B Ford-Lloyd, M Jackson and HJ Newbury. 1995. Use of RAPD for the study of diversity within plant germplasm collections. Heredity 74:170-179.

Widrlechner MP, LD Knerr, JE Staub and K Reitsma. 1989. Biochemical evaluation of germplasm regeneration methods for cucumber, *Cucumis sativa* L. FAO/IBPGR Plant Genet Res Newsletter 88/89:1-4.

Williams CE and PC Ronald. 1994. PCR template-DNA isolated quickly from monocot and dicot leaves without tissue homogenization. Nucleic Acids Res 22:1917-1918.